STUDIES ON THE OXYGEN CONSUMPTION AND WATER METABOLISM OF TURTLE EMBRYOS[1]

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As a result of the early work of Bohr and Hasselbalch and the later experiments of Murray and others there is available a considerable body of information concerning the respiratory exchange of the chick throughout its embryonic life and, although vertebrate groups other than birds have been less extensively investigated, the respiratory characteristics of the embryos of various fishes and amphibians are also fairly well known. Only two investigations of the respiration of reptilian embryos have been reported however, and these deal with but a few stages.

Bohr (1904) carried on some experiments with the eggs of the snake, *Coluber natrix,* and found a decrease in respiration intensity with increasing age. The respiratory quotient as determined by Bohr was about 0.9 at all of the stages studied.

Zarrow and Pomerat (1937) studied four eggs of the Green Snake (*Liopeltis vernalis*) which were in late stages of development and also investigated the respiration of the young after hatching. They report that the absolute gas exchange of the eggs was of the same order of magnitude as that of the young snakes but the respiratory quotient of the hatched animals was significantly greater than that of the embryos.

The present experiments were undertaken with the purpose of providing closely spaced series of determinations of oxygen consumption of the reptilian embryo from the time the egg is laid until after hatching. Some data on changes in weight of the egg during development and on dry substance and fat content at different stages are also presented.

Material and Methods

The eggs used in these experiments were obtained from four species of turtles; the Common Mud Turtle (*Kinosternon subrurum subrurum*), the Snapping Turtle (*Chelydra serpentina serpentina*), the Three-toed Box Turtle (*Terrapene carolina triunguis*), and the Eastern Painted Turtle (*Chrysemys picta picta*). One of these, the Three-toed Box Turtle, is a terrestrial form while the others are primarily aquatic. The Mud Turtles and Painted Turtles were collected from ponds in nearby Maryland, the Snapping Turtles were purchased from a dealer in Wisconsin and the Three-toed Box Turtles were sent to us from Monticello County, Arkansas, through the courtesy of Dr. Delzie Demaree.

Eggs were obtained by killing the adult females and removing the eggs from the oviducts. They were kept on moist cotton in open dishes in an incubator the

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temperature of which varied between 25.0° and 25.5° C. Most of the eggs maintained under these conditions seem to have developed normally although a number of them died during the course of the long period which elapsed before hatching. Dead eggs had a tendency to become moldy on the surface and were, of course, discarded. Moreover it proved possible after some experience to ascertain, by candling the eggs, whether or not living embryos were present and in all experiments except those with very early stages this test was employed. A further check was provided by opening some of the eggs of the experimental series from time to time or by carrying them through to hatching. It is thus possible to state positively that all of the eggs which provided the data on oxygen consumption were living and apparently developing normally.

To obtain some information concerning changes in weight during development, some eggs of each species were weighed at intervals on an analytical balance. In all cases excess moisture was wiped from the surface before weighing and weights were taken to the nearest milligram.

Determinations of the oxygen consumption of eggs and hatched turtles were carried out with Warburg manometers equipped with flasks having about 120 cc. capacity. The CO_2 was absorbed in the customary manner with KOH, the flasks were not shaken and the eggs were kept dry. In all experiments seven flasks were used simultaneously, one serving as a thermobarometer while the other six contained eggs. In the great majority of the experiments only a single egg was introduced into a flask. With very young stages at the beginning of our study, several eggs were used in each flask. Since, however, not all eggs develop normally it was later necessary to reject some of these experiments because, upon opening the eggs at a later date, one or more of a lot used in a single manometer proved to be infertile or abnormal. Since the rate of oxygen consumption of young stages is low, while that of older ones is high, the individual determinations were carried through periods of various lengths. With very young stages the usual period was 24 hours with two or three readings only, with older stages the period was 5 to 6 hours with hourly readings and with late stages 2 to 3 hours with half-hourly readings. In the case of very late stages of the Snapping Turtle the experimental periods were sometimes limited to 1½ hours because the oxygen consumption was so high as to preclude further readings with the manometers used. At the outset of the experiments it became clear that the temperature equilibration between the surroundings and the egg is very slow. When eggs were introduced directly from the incubator at 25.0–25.5° C. into the manometer flasks in a water bath at $25.4 \pm 0.01°$ C. the equilibration took at least two hours. It was therefore necessary to keep the eggs that were to be used for an experiment in the water bath over night. This was done by placing them on moist cotton in an open jar immersed in the water bath. Under such conditions an equilibration of 45 minutes before the manometers were closed proved to be sufficient.

Since it was necessary, in making the calculations of oxygen consumption, to know the volumes of the eggs used, a method was devised for measuring the volumes to the nearest 0.1 cc. The apparatus employed for this [2] was a wide-mouthed, glass-stoppered bottle with a 10 cc. graduated pipette fused in the stopper and a 20 cc. graduated syringe fused in the side of the bottle near its base. The bottle

[2] We are indebted to Mr. W. F. Simpson of this department for devising this apparatus.

was filled with water, closed and the water forced into the pipette by pushing the syringe to the zero mark. A reading was then taken on the pipette. The syringe was opened again to lower the water level, the stopper of the bottle was removed and the egg to be measured was inserted. The top was then replaced, the syringe was pushed to the zero mark and a new reading was taken on the pipette. The difference between this reading and the preceding gives the volume of the egg.

Determinations of the dry substance and fat content were carried out as follows. Two batches of young Snapper eggs (ten eggs each) and two batches of newly hatched Snappers (ten and seven turtles respectively) were dried to constant weight in an oven at 100–105° C. In the case of the eggs, the egg contents and the shells were dried separately. The fat content was determined according to the method of Kumagava and Suto (1908) with the slight modification used by Reinhard and von Brand (1944).

Large numbers of eggs of the Painted Turtle and Snapping Turtle were available and eggs of these forms were opened every few days to give closely staged series of preserved embryos. This makes it possible to correlate the oxygen consumption with both the age and the stage of development in these species. From the Mud Turtle and the Three-toed Box Turtle relatively few eggs were obtained and our series of normal stages for these animals are therefore incomplete.

Results

Developmental rate and incubation period

In view of the paucity of published information concerning the embryology of the turtles used in the present work it is necessary, before discussing our experimental results, to give some general account of the developmental history of these forms.

The normal egg complement of a single female differs markedly in the four species. For the Mud Turtle we had only two adult females and these yielded four and three eggs respectively. Six adult Three-toed Box Turtles were used; four of these had only two eggs each in the oviducts and the other two had three eggs each. In a group of ten Painted Turtles the number of oviductal eggs ranged from two to eight, but most individuals had either five or six eggs and the average number was 5.4. The Painted Turtles used were all about the same size, having carapace lengths of 13.0 to 15.5 cm. Nine Snapping Turtles with carapaces 27.0 to 33.0 cm. long were opened. The smallest number of eggs found was 29, the greatest 61. The average yield for the nine animals was 44.5. This is of some interest in view of the fact that Pope (1939) records for the Snapper that "A female lays from twenty to about forty eggs at one time. Higher numbers (even up to seventy) are often given, but they probably are either sheer estimates or counts made in compound nests." It is, of course, not certain that all of the eggs found in the oviducts would be laid at one time or in one nest but our records do show that a single female may produce well over 40 eggs during one season.

In all of these species, and probably in turtles generally (Risley 1932, 1944), the eggs pass through the cleavage stages while still within the oviduct and are in early stages of gastrulation when laid. If the eggs are retained in the oviduct for unusually long periods they practically cease development during this time, possibly because of anaerobic conditions, and therefore eggs removed directly from the oviduct are all at the same stage even though the dates of removal may be several weeks apart. Thus in our experiments, from a single lot of Snapping Turtles some females were killed on June 30 while others were retained until July 27, but the eggs of both were at the same stage of development when removed and were carried through to hatching with equal success.

At the temperature used in the present work (25.0–25.5° C.) the average times required for incubation were as follows: Mud Turtle, 76 days; Painted Turtle, 63 days; Snapping Turtle, 72 days. None of the Three-toed Box Turtle eggs were carried through to hatching but eggs opened after 70 days incubation contained well-formed turtles attached to yolk masses about 6.0 mm. in diameter. They would probably have required at least 10 more days before hatching. It should be pointed out that even under uniform environmental conditions there is some variation in the rate of development and the period of incubation. For example,

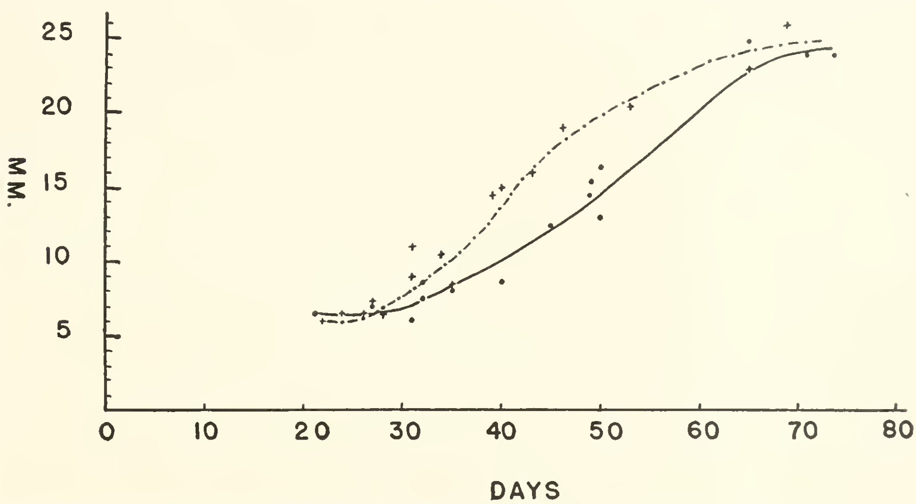

FIGURE 1. The increase in length of the carapace of the turtle embryo during the period of development. ● = Painted Turtle; + = Snapping Turtle.

in a single clutch of 8 Painted Turtle eggs the incubation period varied from 58 to 68 days and in a clutch of 61 Snapping Turtle eggs the limits of variation were 69 to 78 days.

As an indication of the relative rate of growth of the embryo at successive stages, measurements have been made of the series of preserved embryos of the Painted Turtle and the Snapper. Because of the difficulty of making reliable measurements of total length in these animals it has seemed best, for the present purpose, to use the length of the carapace. This can be measured accurately and does seem to furnish a reliable criterion of the rate of growth of the body as a whole. Such a procedure necessitates neglecting about the first twenty days of development, before the carapace is formed, but as will be seen it is the latter part of development which is of most interest in our results on the oxygen consumption.

Figure 1 gives curves for the increase in length of the carapace in the Painted Turtle and Snapping Turtle. Each point represents a single measurement. In the case of the Painted Turtle all embryos in the series were from eggs kept in the incubator. Because of lack of space, the Snapper eggs used for the preserved series were kept in a laboratory the temperature of which varied but averaged about 25° C. The fact that these eggs hatched at the same time as did those in the experimental series of Snapper eggs which were kept in the incubator seems to justify the conclusion that the growth rate in the two lots was quite similar.

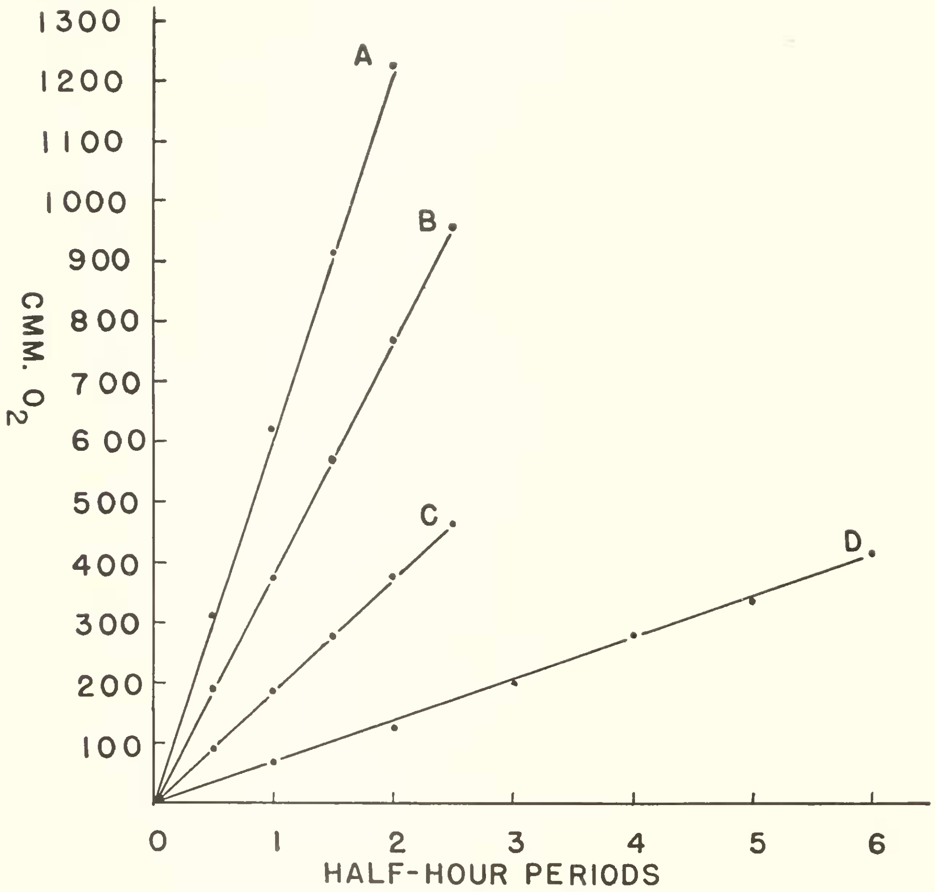

FIGURE 2. Oxygen consumption of four different specimens during consecutive periods of single experiments. A = newly hatched Snapping Turtle; B = Snapping Turtle egg, 42 days incubation; C = Mud Turtle egg, 45 days incubation; D = Painted Turtle egg, 23 days incubation.

It will be noted that the curves for the two species agree closely and indicate that the growth rate is relatively low during the early stages of development but rises rapidly after about the thirtieth day and then tends to remain nearly constant after the sixtieth day. The growth rate of the Snapping Turtle is somewhat higher than that of the Painted Turtle during the period from the thirtieth to fiftieth day however, so that its curve lies at a higher level than does that of the Painted Turtle during this time. The general similarity of these curves to those for oxygen consumption will be noted later.

It should be pointed out that, although the length of the carapace at hatching was found to be the same in the Painted Turtle and Snapping Turtle, the Snapper hatchling is really much the larger animal for it is broader and bulkier than the young Painted Turtle. Thus the average weight of ten new-hatched Painted Turtles was 4.25 grams while the average weight of ten Snapper hatchlings was 7.18 grams. Clearly, therefore, the measurements of carapace length serve only

TABLE I

Oxygen consumption of developing turtle eggs, turtles in the process of hatching (hg) and newly hatched turtles (h). The figures are mean values representing cubic millimeters of oxygen per egg or organism per hour.

Age in days	Three-toed Box Turtle	Mud Turtle	Painted Turtle	Snapping Turtle
1			18	
2	14		32	
9	29	13		
11				35
12		12		
15			39	
16		21		40
19	51			
23		31		55
24	80			
32			95	
34		82		109
35	136			
37			133	
41			185	
42				332
44	180			
45		171		
49			293	
50	287		375	
52				600
54	349	386	526	
56			456	
57			396	
61			463	
62		387	552	798
63	428		632 (hg)	
64			427	
65			476	
69			555	751
70	350	447		1,036 (hg)
72				868 (hg) 641 (h)
73			581	
75		418		617 (h)
76		517 (h)	607 (h)	795 (h)
81			748 (h)	
83		437 (h)		
90		541 (h)		

to give an indication of relative growth rates at different periods and do not tell us the absolute rate of growth. Since, however, our primary interest in relation to the oxygen consumption experiments is in the relative rate of growth, the data derived from the length measurements seem sufficient for our purpose.

Oxygen consumption

When the precautions outlined in the section on material and methods were taken, the successive readings of single manometers proved to be quite regular regardless of the stage of development of the contained egg or young turtle. Examples of the actual oxygen consumption during individual experiments taken at random from our protocols are shown in Figure 2 to illustrate the various lengths of experimental periods, the various times between readings and the obvious regularity of the oxygen consumption.

The mean values of all our experiments on the oxygen consumption of the turtle egg are presented in Table I. The number of individual determinations at each stage varied somewhat but most of the figures in this table are averages of from three to six determinations. These same data are shown graphically in Figures 3 and 4. The figures for the Mud Turtle, Painted Turtle and Three-toed

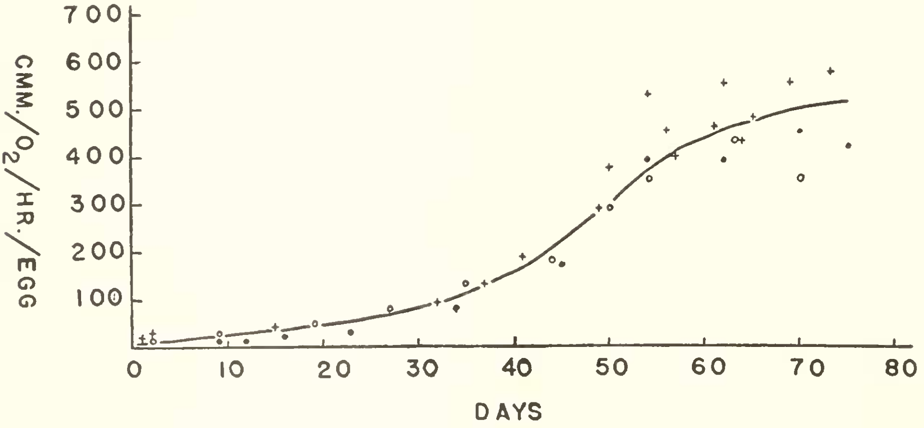

FIGURE 3. Oxygen consumption of the developing eggs of the Mud Turtle, the Painted Turtle and the Three-toed Box Turtle during the period of incubation. ● = Mud Turtle, + = Painted Turtle, ○ = Three-toed Box Turtle.

Box Turtle were fairly close, if identical ages are compared, and they fit rather well to one curve (Fig. 3). The egg of the Snapping Turtle, as was to be expected in view of its greater size, proved to have a much higher oxygen consumption than did the eggs of the other three species (Fig. 4). It has been pointed out that the weight of a newly hatched Painted Turtle is only about 60 per cent of that of a Snapper hatchling and it may be assumed that such weight differences exist even at early embryonic stages. Despite the fact that the curve for the Snapper is at a higher level than that for the other three species, it is obvious that the fundamental nature of the curve is the same in all the forms investigated. Its general shape corresponds to that of the respiratory curves described for the hen's egg (Bohr and Hasselbalch 1900, 1903; Murray 1925). It should be noted that the curve for the oxygen consumption of the Snapper egg shows a somewhat steeper slope during the period between the thirtieth and fiftieth days than does the curve for the other three species. Reference to Figure 1 shows that this must be related to the more rapid increase in size shown by the Snapper embryo during this time. The similarity of the curves for oxygen consumption to those for length increase is quite striking.

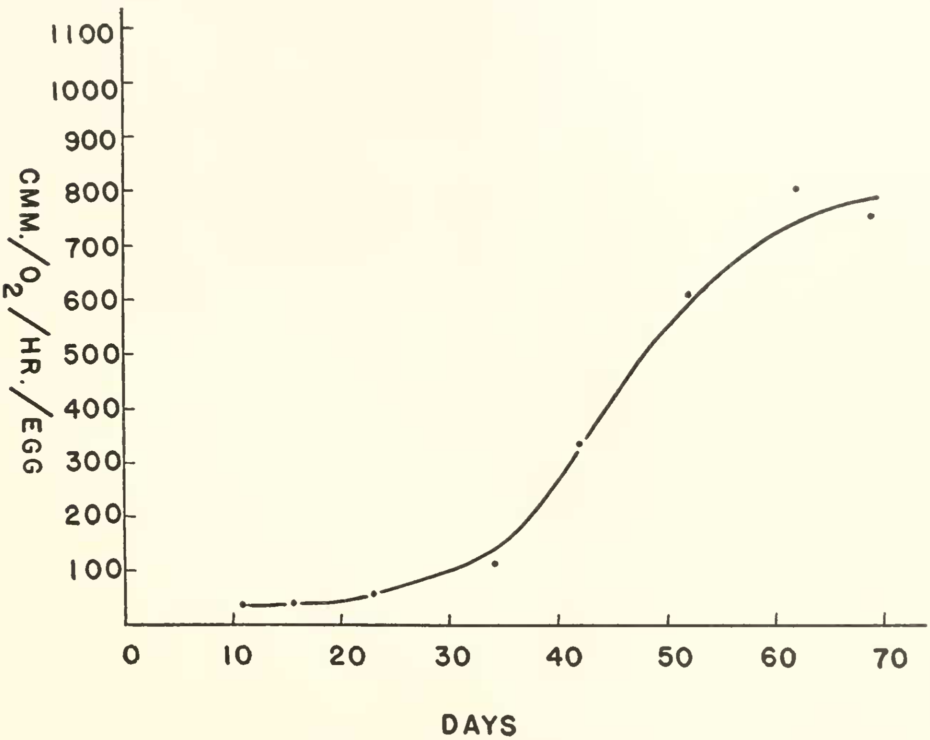

FIGURE 4. Oxygen consumption of the developing eggs of the Snapping Turtle during the period of incubation.

In the case of the turtle one may say that, in general, during the first 50 days of incubation the oxygen consumption is a little less than doubled every 10 days. The single points deviate from the curve but slightly during the first 40 to 50 days but later the deviations become quite pronounced. This period of irregularity corresponds more or less with the period when the curve flattens out. There is, of course, no doubt that the movements of the embryo increase during the latter part of development and it can be assumed that variations in muscular activity are responsible for the variability in the rates of oxygen consumption of older embryos. That this assumption is correct can be inferred from another observation. In Table I are listed some values for turtles that were in the process of hatching and it will be seen that these values are higher than both those for advanced embryos and those for young hatched specimens. The obvious explanation is that the breaking of the egg shell and emergence from the egg require special muscular efforts and so necessitate a higher oxygen consumption during that time.

It should be noted that the values listed in Table I for the oxygen consumption of hatched turtles are about the same as, or only slightly higher than, those for advanced embryos. This is in line with the observation of Zarrow and Pomerat (1937) on embryos and young of the Green Snake. In the case of the chick however the young, during the first ten days, consumes about five times as much oxygen per hour as does the embryo just before hatching (Lussana 1905). This is doubtless to be ascribed to the great amount of muscular activity of the young chick.

The average oxygen consumption per gram per hour for the hatched turtles were as follows: Mud Turtle, 178 cmm.; Painted Turtle, 186 cmm.; Snapping Turtle, 106 cmm. An exact comparison with rates of oxygen consumption of adult turtles is not possible since no data are available from experiments performed

TABLE II

Individual variations in the oxygen consumption of eggs of the same age

Species	Age in days	O_2 consumption in cmm./egg/hour					
Snapping Turtle	24	59	67	39	52	59	
Snapping Turtle	52	806	355	624	592	710	516
Snapping Turtle	62	730	914	553	860	884	845
Three-toed Box Turtle	27	82	73	85			
Three-toed Box Turtle	44	206	153				
Painted Turtle	37	140	144	116			
Painted Turtle	54	493	538	494	565	536	
Painted Turtle	76	645	568	636	581		

near 25° C. Baldwin's (1926) figures for experiments conducted at 20° C. are: Painted Turtle (*Chrysemys picta marginata*) of 1,200 gm., 49.2 cmm./gm./hr.; Snapping Turtle of 1,700 gm., 56.2 cmm./gm./hr. If, as can be supposed, the temperature coefficient of the turtle respiration is in the normal range of about 2, the oxygen consumption per unit weight of young specimens is somewhat higher than that of adults. This would be in agreement with the findings of many investigators for both cold-blooded and warm-blooded animals. In view of the uncertainty of the temperature coefficient it seems premature to try to calculate whether identical values for juvenile and adult specimens would be obtained if the calculations were based on surface area rather than on weight.

In connection with the curves shown in Figures 3 and 4 it is necessary to emphasize that the individual points on the curves represent averages of several determinations and that, in some cases, there were considerable differences in the rate of oxygen consumption of different eggs of the same age. The extent of such variations is indicated by some examples presented in Table II. In most cases we are not able to account for these variations but it is clear that not all eggs developed at exactly the same rate in spite of the uniformity of the external conditions and therefore we occasionally found that two eggs of the same lot, opened on the same day contained embryos which differed significantly in size. For example, two Painted Turtle eggs taken from the same female and incubated for 50 days both contained living and seemingly normal embryos but one embryo measured 13.0 mm. while the other measured 16.5 mm. Similar cases could be cited for the Snapper and the Three-toed Box Turtle. At the later stages of development it is probable that individual differences in the amount of movement of the embryos during the period of the experiment would suffice to account for most of the variations in oxygen consumption. It is, in fact, apparent from our protocols that, during the late stages, a single egg used on successive days often showed a regular, but relatively high rate of oxygen consumption on one day and a regular but low rate on another. There would thus seem to be some periods of activity of fairly long duration and other periods when the embryo is quiescent.

Despite the individual variations discussed above it is clear, from examination of Figures 3 and 4, that the number of determinations and the number of eggs used in these experiments must have been large enough to eliminate any great errors due to variations. Otherwise one would hardly expect such smooth curves.

Changes in weight, dry substance and fat content during development

Previous observations on weight changes in the eggs of turtles have indicated that there is a considerable water intake during development. Cunningham and Hurwitz (1936) report a 37 per cent increase in the weight of the egg of the Diamond-back Terrapin and a 50 per cent increase in the egg of the Marine turtle, *Caretta caretta*. Cunningham and Huene (1938) found that the Snapping Turtle egg may show a maximum weight increase of 60 per cent and the Painted Turtle egg a maximum increase of 75 per cent.

In view of these results Needham (1942) lists the chelonian egg as a non-cleidoic egg, one which is dependent upon uptake of water from the environment for successful incubation. Although this subject was not of immediate concern in the present investigation, some of the eggs of each species were weighed from time to time and the results seem worthy of mention as indicating that the eggs of different species are quite differently constituted in respect to their water requirements and that the amount of water intake necessary for successful development need not be so great as the figures of Cunningham and Huene would indicate. In our experiments the eggs of the Snapping Turtle, the Painted Turtle and the Three-toed Box Turtle all showed an increase in weight during development but the increases were not so great as those reported by Cunningham and Huene. This difference is probably due to a difference in conditions. All of the eggs in our experimental series were kept on cotton which was constantly kept moist and the incubator always had some moisture condensed on the walls. Under these conditions a high percentage of the eggs hatched successfully. On the other hand, eggs kept on moist cotton on a laboratory shelf lost water rapidly, as evidenced by collapsing of the shells, and it was found necessary to keep these eggs covered by a layer of wet cotton in order to bring them through to hatching. The eggs used by Cunningham and Huene were buried in moist sand.

A group of eight Painted Turtle eggs which were kept in the incubator were weighed at approximately weekly intervals throughout their development and the young turtles from these eggs were weighed shortly after hatching. The initial weights, taken within 2 hours after removal from the oviducts, ranged from 4.858 gm. to 5.773 gm. and gave an average of 5.275 gm.; at 60 days incubation the weights ranged from 5.821 gm. to 6.349 gm. and averaged 6.069 gm. There was thus a weight increase of 0.794 gm., approximately 15.1 per cent of the initial weight. It is of interest that this increase was made mostly during the first 9 days of development and that after that time the weights of all eggs stayed fairly constant. The average weight of these eggs 24 hours after removal from the oviducts was 5.286 gm. but at the next weighing (9 days) the average weight was 6.020 gm. The young which hatched from these eggs varied in weight from 3.867 gm. to 4.470 gm. and averaged 4.205 gm.

Our data for the Snapping Turtle and the Three-toed Box Turtle, although not so complete as those for the Painted Turtle, indicate a similar water uptake from the environment. The average weight of ten Snapping Turtle eggs at one day of incubation was 9.944 gm.; at 61 days they had increased in weight to give an average of 10.979 gm., an increase of 10.4 per cent. The average weight of five Three-toed Box Turtle eggs was 8.153 gm. at one day and 9.66 gm. at 70 days, an increase of 18.5 per cent.

Only four eggs of the Mud Turtle were available but these, although they were kept in the same dishes as the Painted Turtle eggs and were weighed at the same times, gave strikingly different results. The four eggs had initial weights of 4.010 to 4.370 gm. and averaged 4.211 grams. The averages for the later weighings were as follows: 9 days, 4.120 gm.; 19 days, 4.144 gm.; 28 days, 4.106 gm.; 38 days, 4.147 gm.; 54 days, 4.120 gm.; 60 days, 4.112 gm. Thus these eggs under the same conditions as those of the Painted Turtle showed no significant change in weight during the whole developmental period. The difference between these two is probably to be attributed to the difference in the nature of the egg shell for the shell of the Mud Turtle egg is hard, thick and brittle while the Painted Turtle egg has a shell which though tough, is more or less parchment-like and can be dented with the finger without breaking. The eggs of the Snapper and Three-toed Turtle have thicker and more rigid shells than do those of the Painted Turtle but do not have the brittle, china-like appearance of Mud Turtle eggs.

In view of the fact that a high percentage of the eggs in our experimental series hatched and gave normal young turtles many of which are still alive we may conclude that the egg of the Mud Turtle is a cleidoic egg in Needham's sense, requiring no uptake of water for its successful development and that the eggs of the other three species used are able to carry through their development with much less water uptake than was previously believed. Just what the minimum requirements may be remains to be investigated.

The initial differences in weight of the eggs of the different species used are, of course, chiefly due to characteristic differences in size of the eggs. In the extensive literature dealing with the natural history of turtles it is customary to indicate these size differences by giving measurements of the length and breadth of the egg but since turtle eggs are often rather irregular in shape we feel that the volume of the egg furnishes a more reliable index of its relative size. The average volumes of the eggs used in the present work were as follows: Mud Turtle, 3.7 cc.; Painted Turtle, 5.3 cc.; Three-toed Box Turtle, 8.1 cc.; Snapping Turtle, 8.7 cc. The limits of individual variation were: Mud Turtle (4 eggs), 3.6–4.0 cc.; Painted Turtle (26 eggs), 4.7–6.1 cc.; Three-toed Box Turtle (12 eggs), 7.1–9.0 cc.; Snapping Turtle (17 eggs), 7.5–9.9 cc. It may be of interest to note that in our largest clutch of Snapping Turtle eggs (61 eggs) there were two which were unusually large, having volumes of 15.3 and 13.5 cc. respectively. These when opened proved to be double-yolked eggs both of which were, unfortunately, infertile. We have seen no other double-yolked turtle eggs nor have we encountered any reference to them in the literature.

Our data on the changes in dry substance and in fat content apply to the Snapping Turtle only and were derived from determinations on 20 eggs (at 15 days incubation) and on 17 newly hatched turtles. The eggs were analyzed in two batches of ten eggs each and the turtles in two batches of ten and seven animals respectively. The results of these determinations are summarized in Table III.

TABLE III

Changes in dry substance, water content and ether extract during the development of the Snapping Turtle egg. The values are expressed in grams per egg (or, in the case of hatched turtles, in grams per individual) and are mean values of two determinations. The single values are given in parentheses.

	Egg contents	Egg shell	Hatched turtles	Decrease during development
Fresh weight	8.80 (8.54, 9.05)	1.56 (1.56, 1.57)	7.36 (7.54, 7.18)	1.44
Dry weight	1.91 (1.89, 1.92)	1.14 (1.15, 1.13)	1.45 (1.52, 1.38)	0.46
Water	6.89 (6.65, 7.13)	0.42 (0.41, 0.44)	5.91 (6.02, 5.80)	0.98
Ether extract	0.273 (0.258, 0.288)		0.212 (0.220, 0.203)	0.061

These data show that the hatched animal has considerably less water than the original egg and about 0.5 gram less dry substance. Much of the water loss is to be accounted for by the fluids of the amniotic and allantoic cavities which are lost at hatching. We have no precise measurements on this for the Snapping Turtle but one of the Mud Turtle eggs was opened on the 77th day and the following weights were obtained: whole egg, 4.341 grams; embryo, 3.207 grams; shell, 0.674 gram; fluid, 0.409 gram; unaccounted for, 0.054 gram. The fluid lost at hatching thus amounted to at least 9.4 per cent of the weight of the whole egg just before hatching. A comparable percentage is undoubtedly lost in the Snapping Turtle. Although no determinations of the inorganic substances were made, it can be assumed that the loss of dry substance was primarily due to the oxidation of organic material.

Fat was consumed to the extent of 61 mg. per egg. Since one gm. of fat of average composition requires 2,020 cc. of oxygen for total oxidation the fat disappearing from the Snapper egg would require 123 cc. of oxygen for its combustion. The total oxygen consumption during development up to hatching, as computed from Figure 4, is about 500 cc. This leaves about 380 cc. of oxygen for oxidation of the 400 mgm. of dry substance additionally consumed (total dry substance used, minus fat consumed). Four hundred mgm. of carbohydrate are totally oxidized by 300 cc. of oxygen and the same amount of protein by 400 cc. of oxygen. It